

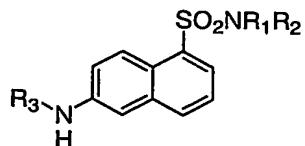
What is claimed is:

1. A method for determining tissue factor (TF) activity in a sample suspected to contain TF, comprising:

- 5 (a) combining TF and a molar excess of factor VIIa (fVIIa) to produce a TF/fVIIa enzyme complex; and
 (b) detecting enzymatic activity of the complex using a fluorogenic or chromogenic substrate.

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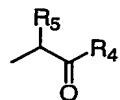
2. The method of claim 1, wherein the substrate is a compound of the formula:



15 or a pharmaceutically acceptable non-toxic salts thereof; wherein

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R_1 is hydrogen, straight or branched chain lower alkyl having 1-6 carbon atoms optionally substituted with $\text{C}_1\text{-C}_6$ alkoxy, straight or branched chain alkenyl having 2-8 carbon atoms, straight or branched chain alkynyl having 2-8 carbon atoms, cycloalkyl having 3-7 carbon atoms, alkylcycloalkyl where the alkyl portion has 1-6 carbon atoms, cycloalkylakyl where the alkyl portion has 1-6 carbon atoms, or phenylalkyl where the alkyl portion is straight or branched chain alkyl having 1-6 carbon atoms, or a group of the formula



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R_5 represents hydrogen or an amino acid side chain; and

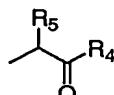
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R_4 is hydroxy, $\text{C}_1\text{-C}_6$ alkoxy, an amino acid or a peptide residue;

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R₂ is hydrogen, straight or branched chain lower alkyl having 1-6 carbon atoms, straight or branched chain alkenyl having 2-8 carbon atoms, straight or branched chain alkynyl having 2-8 carbon atoms, cycloalkyl having 3-7 carbon atoms, alkylcycloalkyl where the alkyl portion has 1-6 carbon atoms, or phenylalkyl where the alkyl portion is straight or branched chain alkyl having 1-6 carbon atoms, or a group of the formula

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R₅ represents hydrogen or an amino acid side chain; and

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R₄ is hydroxy, C₁-C₆ alkoxy, an amino acid or peptide residue; or

NR₁R₂ forms a nitrogen heterocycle; and

R₃ is an amino acid or a peptide residue.

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3. The method of claim 1, where the substrate is a chromogenic substrate.

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4. The method of claim 3, where the chromogenic substrate is a para-Nitroaniline-based substrate.

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5. The method of claim 1, further comprising (c) generating a numerical value associated with the enzymatic activity of the sample and (d) comparing the numerical value with a standard curve of TF-dependent enzymatic activity.

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6. The method of claim 5, wherein the standard curve is generated by quantifying TF-dependent enzymatic activity of the TF/fVIIa complex in samples with known concentrations of TF.

7. The method of claim 6, wherein the TF is native human tissue factor.

8. The method of claim 6, wherein TF source is brain tissue, placenta, endothelial cells, tissue extract, plasma, cell extract, synthetic or naturally derived thromboplastin, or
5 recombinant human tissue factor.

9. The method of claim 6, wherein the fVIIa is native human factor VIIa or recombinant factor VIIa.

10 10. The method of claim 6, wherein the TF and the fVIIa are not of human origin.

11. The method of claim 6, wherein the concentration of TF is from 0.1 pM to 1mM.

15 12. The method of claim 6, wherein the reaction mixture contains divalent metal ion or a metal ion chelator.

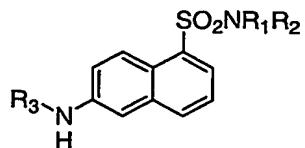
20 13. The method of claim 12, wherein the divalent metal ion is calcium ion, magnesium ion or manganese ion.

25 14. The method of claim 12, wherein the metal ion chelator is ethylenediaminetetraacetic acid (EDTA) or ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA).

15. A method for determining factor VIIa (fVIIa) enzymatic activity in a sample suspected to contain fVIIa, comprising:

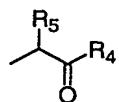
30 (a) combining fVIIa and a molar excess of TF to produce a TF/fVIIa enzyme complex; and
(b) detecting the enzymatic activity of the complex using a fluorogenic substrate or a chromogenic substrate.

16. The method of claim 15, wherein the substrate is a compound of the formula:



or a pharmaceutically acceptable non-toxic salts thereof;
5 wherein

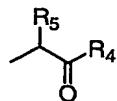
R₁ is hydrogen, straight or branched chain lower alkyl having 1-6 carbon atoms optionally substituted with C₁-C₆ alkoxy, straight or branched chain alkenyl having 2-8 carbon atoms, straight or branched chain alkynyl having 2-8 carbon atoms, cycloalkyl having 3-10 carbon atoms, alkylcycloalkyl where the alkyl portion has 1-6 carbon atoms, cycloalkylakyl where the alkyl portion has 1-6 carbon atoms, or phenylalkyl where the alkyl portion is straight or branched chain alkyl having 1-6 carbon atoms, or a group of the formula
15



R₅ represents hydrogen or an amino acid side chain;
20 and

R₄ is hydroxy, C₁-C₆ alkoxy, an amino acid or a peptide residue;

R₂ is hydrogen, straight or branched chain lower alkyl having 1-6 carbon atoms, straight or branched chain alkenyl having 2-8 carbon atoms, straight or branched chain alkynyl having 2-8 carbon atoms, cycloalkyl having 3-25 carbon atoms, alkylcycloalkyl where the alkyl portion has 1-6 carbon atoms, or phenylalkyl where the alkyl portion is straight or branched chain alkyl having 1-6 carbon atoms, or a group of the formula
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R₅ represents hydrogen or an amino acid side chain;
and

R₄ is hydroxy, C₁-C₆ alkoxy, an amino acid or peptide residue; or

5 NR₁R₂ forms a nitrogen heterocycle; and
R₃ is an amino acid or a peptide residue.

17. The method of claim 15, where the substrate is a chromogenic substrate.

10 18. The method of claim 17, where the chromogenic substrate is a pNA-based substrate.

15 19. The method of claim 15, further comprising (c) generating a numerical value associated with the enzymatic activity of the sample and (d) comparing the numerical value with a standard curve.

20 20. The method of claim 19, where the curve is generated by quantifying fVIIa-dependent enzymatic activity of the TF/fVIIa complex in samples with known concentrations of fVIIa.

25 21. The method of claim 19, wherein the fVIIa is native human factor VIIa or recombinant human factor VIIa.

22. The method of claim 19, wherein the fVIIa source is plasma, tissue extract, cell extract or recombinant material.

30 23. The method of claim 19, wherein the TF is native human tissue factor or recombinant human tissue factor.

24. The method of claim 19, wherein the TF source is a synthetic or naturally derived thromboplastin.

35 25. The method of claim 19, wherein the fVIIa and the TF are not of human origin.

26. The method of claim 19, wherein the concentration of fVIIa is from 0.1 pM to 1mM.

5 27. The method of claim 19, wherein the reaction mixture contains divalent metal ion or a metal ion chelator.

28. The method of claim 27, wherein the divalent metal ion is calcium ion, manganese ion or magnesium ion.

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29. The method of claim 27, wherein the metal ion chelator is ethylenediaminetetraacetic acid (EDTA) or ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA).